REMARKS

Status of the claims

Claims 21, 28, 40, 43, 99-104, 106-113 and 119-143 were pending. Claims 43, 109-113 and 119-143 remain withdrawn pursuant to the Restriction Requirement and claims 21, 28, 40, 99-104 and 106-108 are under active examination. By amendment herein, claims 21, 28, 40, 43, and 127 have been amended to specify that the DNA-binding domain is a zinc finger DNA-binding domain. Accordingly, claims 106, 119 and 136 have been canceled, without prejudice or disclaimer. Claims 28, 40, 43 and 127 have also been amended to clarify that the chromosomal DNA is endogenous chromosomal DNA. See, e.g., Example 3 of specification. Typographical errors in claims 126 and 143 have also been corrected.

Thus, claims 21, 28, 40, 43, 99-104, 107-113, 120-135, and 137-143 are pending and claims 21, 28, 40, 99-104 and 107-108 are under active examination.

Applicants reiterate that rejoinder of the method claims is in order upon indication of allowable composition claims.

35 U.S.C. § 112, first paragraph, written description

Previous claims 21, 28, 40, 99-104 and 106-108 were rejected under 35 U.S.C. § 112, 1st paragraph as allegedly failing to comply with the written description requirement. (Office Action, paragraph 5). The rejection is premised on several allegations, including that there is only a single representative species disclosed, that the claimed genus contains "structurally unrelated members" and that "neither the specification nor the art teach the structural features required in a DNA-binding domain such that it can recognize any DNA target." *Id.* In addition, Berglund et al. was cited as allegedly showing unpredictability of DNA-binding domains. *Id.*

To the extent that the foregoing claim amendments do not obviate the rejection, Applicants traverse the rejection and supporting remarks.

The fundamental factual inquiry in written description is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed. *See, e.g., Vas-Cath, Inc.*, 935 F.2d at 1563-64, 19 USPQ2d at 1117. Determining whether the written description requirement is

satisfied is a question of fact and the burden is on the Examiner to provide evidence as to why a skilled artisan would not have recognized that the applicant was in possession of claimed invention at the time of filing. *Vas-Cath, Inc. v. Mahurkar*, 19 USPQ2d 1111 (Fed. Cir. 1991); *In re Wertheim,* 191 USPQ 90 (CCPA 1976). It is not necessary that the application describe the claimed invention *in ipsis verba.* Rather, all that is required is that the specification reasonably convey possession of the invention. *See, e.g., In re Lukach,* 169 USPQ 795, 796 (CCPA 1971). Finally, determining whether the written description requirement is satisfied requires reading the disclosure in light of the knowledge possessed by the skilled artisan at the time of filing, for example as established by reference to patents and publications available to the public prior to the filing date of the application. *See, e.g., In re Lange,* 209 USPQ 288 (CCPA 1981).

Furthermore, the Patent Office's own guidelines on written description are clear -- the written description requirement is highly fact-dependent and there is a strong presumption that an adequate written description of the claimed invention is present at the time of filing:

> [t]he description need only describe in detail that which is new or not conventional. This is equally true whether the claimed invention is a product or a process. An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that the applicant was in possession of the claimed invention, i.e. complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with known or disclosed correlation between function and structure, or some combination of such characteristics. (Final Examiner Guidelines on Written Description, 66 Fed. Reg. 1099, emphasis added).

Applying these rules to the case at hand, it is clear that the as-filed specification, in light of the art available at the time of filing, describes the claimed genus of compositions comprising a chimeric nuclease that includes a zinc finger DNA-binding domain.

The pending claims are drawn to compositions which necessarily include a zinc finger DNA-binding domain. Contrary to the Examiner's assertion, zinc finger DNA-binding domains are structurally related. The specification clearly sets forth the structure of these proteins, which includes a backbone region and one or more recognition helices that are involved in DNA binding. See, e.g., paragraphs [0092] and [0100] of the as-filed specification and references

cited therein. Likewise, the state of the field at the time of filing clearly demonstrates that the skilled artisan knew that zinc finger DNA-binding domains were structurally related: (See, e.g., U.S. Patent No. 6,534,261 (Issued March 18, 2003; Reference AQ of IDS mailed on February 13, 2004) at column 2, lines 43-60):

> Zinc finger proteins ("ZFPs") are proteins that can bind to DNA in a sequence-specific manner. Zinc fingers were first identified in the transcription factor TFIIIA from the oocytes of the African clawed toad, Xenopus laevis. ZFPs are widespread in eukaryotic cells. An exemplary motif characterizing one class of these proteins (C_2H_2 class) is -Cys-$(X)_{2-4}$ -Cys-$(X)_{12}$ -His-$(X)_{3-5}$ -His (SEQ ID NO:1 (where X is any amino acid). A single finger domain is about 30 amino acids in length and several structural studies have demonstrated that it contains an alpha helix containing the two invariant histidine residues co-ordinated through zinc with the two cysteines of a single beta turn. To date, over 10,000 zinc finger sequences have been identified in several thousand known or putative transcription factors. ZFPs are involved not only in DNA-recognition, but also in RNA binding and protein-protein binding. Current estimates are that this class of molecules will constitute about 2% of all human genes.

Moreover, the specification and state of the art clearly teach that these structurally-related zinc finger DNA-binding domains can be modified in their recognition helices so as to recognize any selected target site (see, e.g., paragraphs [0100] and [0165] of the as-filed specification):

> Various methods for designing chimeric nucleases with varied DNA recognition sequences are known in the art. In certain embodiments, the DNA binding domain comprises one or more zinc finger domains (or referred to as zinc fingers). The zinc fingers can be engineered to recognize a selected target site in the target sequence. As described above, Cys_2His_2 proteins may be engineered to recognize a wide variety of sites. As another example, zinc fingers can be selected by using polypeptide display libraries. The target site is used with the polypeptide display library in an affinity selection step to select variant fingers that bind to the target site. Typically, constant zinc fingers and fingers to be randomized are made from any suitable C_2H_2 zinc finger protein, such as SP-1, SP-1C, TFIIIA, GLI, Tramtrack, YY1, or

> ZIF268 (see, e.g., Jacobs, EMBO J. 11:4507 (1992); Desjarlais & Berg, Proc. Natl. Acad. Sci. U.S.A. 90:2256-2260 (1993)). The polypeptide display library encoding variants of a zinc finger protein comprising the randomized finger, one or more variants of which will be selected, and, depending on the selection step, one or two constant fingers, is constructed according to the methods known to those in the art. Optionally, the library contains restriction sites designed for ease of removing constant fingers, and for adding in randomized fingers. Fingers are randomized, e.g., by using degenerate oligonucleotides, mutagenic cassettes, or error prone PCR. See, for example, U.S. Pat. Nos. 6,326,166, 6,410,248, and 6,479,626.
>
> Chimeric nucleases are modular in nature with the DNA binding specificity residing in the zinc finger domain. By modifying the DNA binding specificity of the zinc finger domain, they can be engineered and optimized to bind specifically to a wide variety of nine bp sequences (Rebar et al., 1994, Science, 263:671-3; Wolfe et al., 2001, Structure (Camb), 9:717-23; Sera and Uranga, 2002, Biochemistry, 41:7074-81). Thus, one should be able to engineer chimeric nucleases to stimulate gene targeting at any locus.

The state of the art also evidences that, at the time of filing, the skilled artisan knew how to alter the structure of any zinc finger DNA-binding domain so as to bind to any pre-selected target site (See, U.S. Patent No. 6,534,261 (Issued March 18, 2003; Reference AQ of IDS mailed on February 13, 2004) at column 15, lines 7-17):

> The ZFPs of the invention are engineered to recognize a selected target site in the endogenous gene of choice. Typically, a backbone from any suitable C2H2 ZFP, such as SP-1, SP-1C, or ZIF268, is used as the scaffold for the engineered ZFP (see, e.g., Jacobs, EMBO J. 11:4507 (1992); Desjarlais & Berg, PNAS 90:2256-2260 (1993)). A number of methods can then be used to design and select a ZFP with high affinity for its target (e.g., preferably with a Kd of less than about 25 nM). As described above, a ZFP can be designed or selected to bind to any suitable target site in the target endogenous gene, with high affinity.

See, also, U.S. Patent No. 6,013,453 (Issued January 11, 2000; Reference AK of IDS mailed on February 13, 2004) at column 2, lines 48-54:

> Protein engineering experiments have shown that it is possible to alter rationally the DNA-binding characteristics of individual zinc fingers when one or more of the α-helical positions is varied in a number of proteins [references omitted].

Methods of engineering zinc finger DNA-binding domains to bind to any selected target site are also described, in detail, in WO 01/66717 (reference BP of IDS filed February 10, 2006); WO 01/40798 (reference BQ of IDS filed February 10, 2006); and WO 02/04488 (reference BR of IDS filed February 10, 2006).

Thus, the specification and references of record clearly establish that the common structural features to be modified in a zinc finger DNA-binding domain such that it can recognize any selected DNA target were well known at the time of filing. Specifically, the structure of zinc finger proteins, as well as how to alter this structure (amino acid sequence of the recognition helices) to obtain zinc finger proteins that bind to a selected target site are amply described in the specification and art.

Furthermore, the specification and evidence of record completely belies the contention that Berglund et al. somehow establishes that the structure-function relationship of zinc finger DNA-binding proteins was not known at the time of filing. As a threshold matter, it is first noted that Berglund was published in 1997 and, accordingly, is not indicative of the state of the art regarding engineering zinc finger DNA binding domains at the time of filing. Moreover, contrary to the Examiner's assertion, Berglund actually provides evidence that the amino acid residues of zinc finger DNA binding proteins involved in binding DNA were known and that alteration of these residues was known to affect binding. Plainly, this reference further supports that the skilled artisan was aware, at the time of filing, of the structure of zinc finger DNA binding proteins.

In sum, the evidence of record clearly establishes that the as-filed specification fully describes zinc finger DNA binding proteins as claimed. The description in the specification and art clearly establishes that Applicants were in possession of a chimeric nuclease including any zinc finger DNA binding domain. Therefore, the basis of the rejection is unfounded and the rejection should be withdrawn.

35 U.S.C. § 112, first paragraph, enablement

Previous claims 21, 28, 40, 99-104 and 106-108 were rejected under 35 U.S.C. § 112, 1st paragraph as allegedly not enabled by the as-filed specification. (Office Action, paragraph 6). As with written description, it was asserted that the specification does not enable the genera of any DNA-binding domain and/or non-isolated cells. *Id.* Various references are cited for showing the alleged unpredictability of modifying DNA binding domains. *Id.*

Applicants traverse the rejection.

As set forth in the seminal case of *In re Marzocchi,* 439 F.2d, 220, 223, 169 USPQ 367, 369 (CCPA 1971), a patent application is presumptively enabled when filed:

> [a]s a matter of Patent Office practice ... a specification .. must be taken as in compliance with the enablement requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

Moreover,

> it is incumbent upon the Patent Office, whenever a rejection on [grounds of enablement] is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

439 F.2d at 224, 169 USPQ at 369-370. Indeed, as pointed in the Patent Office's own Training Manual on Enablement (1993, citing *In re Wright,* 999 F.2d 1557, 1561-1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993), "the case law makes clear that properly reasoned and supported statements explaining any failure to comply with section 112 are a requirement to support a rejection."

Zinc Finger DNA Binding Domains

In the instant case, the Examiner has not presented a properly reasoned and supported basis for the rejection. However, in the interest of advancing prosecution, the current claims

have been modified to a chimeric nuclease comprising a zinc finger DNA binding domain. For the reasons presented above with regard to written description, the specification and evidence of record clearly establish that the structure-function relationship of zinc finger DNA binding domains was known at the time of filing. The specification teaches precisely how to make an engineering a zinc finger protein to bind to any selected target site and how to use that zinc finger DNA binding domain in a chimeric nuclease.

Moreover, the references cited by the Examiner do not in any way establish lack of enablement of the claimed subject matter. Branden et al. was published in 1991, fully 12 years before the effective filing date of the instant application. In addition, this general reference regarding protein engineering is not relevant to the pending claims. As addressed above, Berglund was also published well prior to Applicants' priority date and is not representative the state of the art regarding zinc finger DNA binding domains at the time of filing. Nor does Berglund teach that binding of zinc finger DNA binding domains to their target sites was unpredictable, but, rather, that the GR residues involved in binding DNA were known and that altering these residues altered binding specificity.

For its part, when properly viewed as a whole, Porteus actually teaches that the designing of zinc finger proteins was routinely performed. The fact that refinements may be desirable for improved discrimination does not establish unpredictability. Similarly, the passage on page 972 cited by in the Office Action does not state that all zinc finger proteins are toxic, but, instead that refinements may be needed for "potentially" toxic ZFNs. Plainly, a notation that "refinements" may be desirable indicate that engineering of zinc finger proteins was, in fact, routine. Simply put, stating that refining zinc fingers in terms of discrimination and specificity is "not trivial" does not show that the zinc finger nucleases are non-functional – the suggestion that improvements may be desirable does not support a non-enablement rejection. In fact, arguments relating to refinement of ZFN characteristics are, as a whole, irrelevant to the claims in the instant invention. The claims do not contain a "non-toxicity" element or limitation, and thus a discussion of Porteus' assertion about potential ZFN toxicities has no bearing on the claims currently under consideration.

In sum, in light of the actual scope of the claims, the nature of the guidance provided in the specification, the conventional nature of engineered zinc finger proteins and making using them to make chimeric zinc finger nucleases, all establish that the specification is fully enabling.

In vivo Methods

With regard to *in vivo* cells (claim 28 and claims dependent therefrom), the Examiner asserts that gene therapy is unpredictable and sites various references (Porteus, Phillips, Gardlik and Urnov) as allegedly supporting unpredictability of the claimed cells on the grounds that "the art teaches the high unpredictability of delivering DNA to human tissues and achieving the desired expression." (Office Action, paragraph 6).

However, the pending claims are not directed to gene therapy methods. Instead, they are directed to specific cells comprising fusion proteins of zinc fingers and a cleavage domain. No evidence has been presented by the Office supporting the assertion that methods involving zinc finger fusion proteins only function in isolated cells. Thus, the arguments relating to the viability of DNA delivery methods are irrelevant.

The as-filed specification clearly teaches that the cells expressing zinc finger nucleases may be isolated or *in vivo*. See, paragraphs [0017], [0134] and [0150]. Moreover, the ability of fusion proteins including an engineered zinc finger protein and a functional domain to be expressed and to regulate gene expression *in vivo* (when introduced as a protein or as a polynucleotide which is then expressed in the cell) is well established. See, U.S. Patent No. 6,534,261 (Issued March 18, 2003; Reference AQ of IDS mailed on February 13, 2004). Thus, there is no basis for the rejection and it should be withdrawn.

Turning to the references cited for allegedly showing unpredictability of cells *in vivo*, Applicants first note that Phillips and Gardlik are in no way concerned with cells comprising zinc finger proteins and, accordingly, do not in any way establish unpredictability of the claimed cells. For their parts, Porteus and Urnov clearly teach that zinc finger nucleases are expected to be expressed (and functional) *in vivo* – both references teaching that chimeric nucleases had been successfully used to targeting frequencies of up to 20% in a human disease-causing gene. Noting that "further work" may be required for *in vivo* applications is a far cry from establishing

unpredictability of cells that express the zinc finger nuclease in such a way as to result in target cleavage.

It is also noted that the Examiner's assertion that Urnov's statements regarding safety concerns such as "potential imunogenicity" somehow establish unpredictability are completely untenable. Indeed, the courts have consistently held that safety is not a criterion of patentability and is irrelevant to an enablement inquiry. See, *In re Krimmel,* 130 USPQ 215 (CCPA 1961); *In re Hartop* 135 USPQ 419 (CCPA 1962); *In re Anthony,* 162 USPQ 594 (CCPA 1969). The criteria for enablement is showing how to make and use the claimed cells and Urnov's demonstration that the frequencies of homologous recombination at an endogenous chromosomal sequence in both cultured cell lines and primary cells were thousands of times higher than those obtained using previous methods clearly establishes that, in cells, zinc finger nucleases would cleave their targets.

Applicants also remind the Office that it is well settled that time-consuming or expensive experimentation is **not** undue if it is routine. (See, *e.g.,* PTO Training Manual on Enablement, pages 30-31, citing *United States v. Telectronics Inc.,* USPQ2d 1217, 1223 (Fed. Cir. 1988), *cert. denied* 490 U.S. 1046 (1989) holding the disclosure of a single exemplified embodiment and a method to determine other embodiments was enabling, even in the face of evidence that determining additional embodiments might require 6-12 months of effort and cost over $50,000). Similarly, the potential presence of inoperative embodiments does not establish lack of enablement. *Atlas Powder Co. v. E. I. duPont de Nemours & Co.,* 224 USPQ 409 (Fed. Cir. 1984).

For the reasons set forth herein, any experimentation needed to practice the claimed methods *in vivo* is routine in view of the teachings of the specification (including working examples) and the state of the art.

Thus, the Office has not provided sufficient evidence supporting non-enablement and, in the absence of necessary relevant evidence contradicting the teachings of the specification and state of the art, the rejection cannot be maintained.

35 U.S.C. § 103(a)

Claims 21, 28, 40, 99-104, and 106-108 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over U.S. Patent Publication No. 20020107214 (hereinafter "Choulika") in view of Bibikova et al. (2001) *Mol. Cell. Biol.* 21:289-297 (hereinafter "Bibikova") and further in view of Takeuchi et al. (2002) *Biochem. Biophys. Res. Commun.* 293:953-957 (hereinafter "Takeuchi"). (Office Action, paragraph 9).

The pending claims are directed to vectors encoding both a chimeric nuclease and a repair substrate as well as to cells in which the repair substrate targets endogenous chromosomal DNA. Choulika, alone or in combination with Bibikova and Takeuchi, fails to teach or suggest such vectors or cells.

With regard to the vectors (claim 21 and claims dependent thereon), Choulika teaches that the nuclease and the repair substrate are carried on separate vectors. Bibikova does not describe or demonstrate a vector as claimed carrying both nuclease and substrate and, in the speculation regarding gene targeting methods, this reference also teaches that the linear donor DNA should be introduced separately from the nuclease-encoding vector. See, Bibikova, page 296, left column. Takeuchi is similarly silent as to vector including sequences encoding both nuclease and repair substrate. Thus, there is no combination of the references that teaches or suggest the subject matter of claims 21, 99-102 and 104.

There is also no combination of the cited references that teaches cells as set forth in claims 28, 40, 103, 107 and 108. In particular, in the interest of advancing prosecution, the claims have been modified to specify that the target sequence is contained in endogenous chromosomal DNA. By contrast, Choulika and Bibikova both fail to teach or suggest cleavage of a chromosomal target. As noted in paragraph in paragraph [0160] of Applicants' specification, Choulika teaches that because the target site for SceI occurs so infrequently in the genome, the target site must be inserted into the genome prior to cleavage (see, also, Choulika, paragraph [0025], emphasis added; see, also Example 2 of Choulika showing generation of cell lines with the SceI non-endogenous target sites):

> [0025] A model chromosomal loci was generated in which a site for the meganuclease I-SceI was introduced within the target

> region for recombination, and double stranded DNA cleavage via introduction of a vector encoding the restriction endonuclease was induced.

Thus, since Choulika relies on introduction of a target sequence, this reference in no way discloses cells in which endogenous chromosomal DNA is targeted.

Nor does Bibikova teach cells in which a zinc finger nuclease cleaves an endogenous chromosomal target, as required. Indeed, Bibikova specifically teaches that the target sequence for the naturally occurring ZFN is "microinjected" into oocytes (see, Bibikova, page 290, paragraph bridging left and right columns, emphasis added; see, also, Fig. 1(B) showing that target DNA is injected into oocytes):

> Here we characterize the cleavage abilities of the chimeric nuclease in *Xenopus laevis* oocytes. These enormous cells have a large capacity for homologous recombination that is readily accessed by microinjection of appropriate substrates ...

Therefore, Bibikova is not cleaving an endogenous chromosomal gene, but a non-endogenous substrate injected into a haploid oocyte.

Indeed, the investigative team including Bibikova clearly chose not to cleave endogenous chromosomal DNA because they were unsure if zinc finger nucleases would result in targeting of endogenous chromosomal DNA by a repair substrate (rather than, for example, non-homologous end joining) (see, Bibikova page 296, right column, emphasis added):

> Several additional issues remain to be addressed to confirm the utility of chimeric nucleases as tools for gene targeting. Among these are demonstrating discrimination against related sequences; proving the efficacy of zinc fingers designed to bind arbitrarily chosen sequences; and testing the cleavage of genuine **chromosomal** targets. The question of discrimination among potential binding sites is a particularly critical one. In this regard, neither QQR nor QNK is the ideal model enzyme, since both can bind alternative sites (23, 29, 51). Impressive zinc finger binding selectivity has been achieved recently with the assistance of negative selection against closely related base triplets (49). An additional concern is the existence of nonhomologous recombination pathways, which will compete with homologous recombination to repair the broken target. It may be possible to take advantage of differences in the genetic requirements of these processes (21, 32, 40) to tip the balance in favor of homologous events.

Furthermore, it is clear that Takeuchi's disclosure of Flp recombinase does not cure the deficiencies of Choulika and Bibikova regarding cells in which the repair substrate targets endogenous chromosomal DNA.

Thus, there is no combination of Choulika, Bibikova and Takeuchi that places the public in possession of vectors encoding a chimeric nuclease and carrying a repair substrate. Nor is there any combination of these references that teaches or suggests cells in which the repair substrate targets endogenous chromosomal DNA. Takeuchi relies on crossing a transgenic mouse line carrying the Flp recombinase transgene to a mouse line carrying an engineered neomycin phosphotransferase gene flanked by Flp target sites. This reporter construct is clearly not an endogenous chromosomal DNA target. Therefore, the rejections should be withdrawn.

CONCLUSION

Reconsideration of the application is requested in light of the foregoing amendments and remarks. If the Examiner notes any further matters that the Examiner believes may be expedited by a telephone interview, the Examiner is requested to contact the undersigned.

Respectfully submitted,

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By: _____
Dahna S. Pasternak
Registration No. 41,411
Attorney for Applicants

ROBINS & PASTERNAK LLP
1731 Embarcadero Road
Suite 230
Palo Alto, CA 94306
Tel. (650) 493-3400
Fax. (650) 493-3440